INDUCTION AND REGULATION OF EXPERIMENTAL UVEITIS

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ANTIGENIC MIMICRY BETWEEN AN HLA-CLASS I-PEPTIDE AND THE MOST UVEITOGENIC PEPTIDE FROM RETINAL S-ANTIGEN

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Purpose: The immunological mechanism responsible for the statistical associations of HLA-antigens and autoimmune uveitis was unknown for many years. Also the location of the induction of the retina-specific immune response was discussed, for the target autoantigens are found exclusively in immune privileged sites. We described a peptide derived from the sequence of disease-associated HLA-antigens, which shares five amino acids with the most uveitogenic region of retinal S-Antigen (S-Ag) and investigated its immunological properties in experimental autoimmune uveitis (EAU) with respect to induction and suppression by oral tolerance.
Methods: Lewis rats were immunized with peptide ALNEDLSSWTADDT (B27PD) from the sequence of HLA-B to induce experimental uveitis. A similar peptide from non-associated HLAs, ALNEDLRSWTAADT (B7PD), was used as control. The same peptides were applied orally to elicit tolerance against disease induced with S-Ag, the corresponding S-Ag-peptide and interphotoreceptor retinoid binding protein (IRBP). Lymphocytes from uveitis patients were tested for proliferation to the HLA- and retinal peptides and proteins.
Results: Peptide B27PD induced a mild uveitis after immunization, while B7PD did not induce disease at all. In contrast to B7PD, Peptide B27PD, when applied orally, even protected from uveits induced with S-Ag, S-Ag peptide, and – to a certain extend – also from IRBP-induced uveitis. The suppressive effect of B27PD even exceeded that of S-Ag or the S-Ag peptide PDSAg. Patient's lymphocytes proliferated to peptide B27PD as well as to S-Ag and the S-Ag-peptide, but not to B7PD.
Conclusions: We postulate antigenic mimicry between the HLA-peptide in the periphery and the sequestered S-Ag peptide within the retina, raising a crossreactive T-cell response as the initiating event for uveitis. This hypothesis can explain the associations of certain HLA-antigens with disease, and an induction of the retina-protein specific autoimmune response in the periphery. At present we are initiating a trial for therapy of uveitis patients by oral tolerance induction with peptide B27PD.
Patent pending.

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THE ROLE OF GAMMA-DELTA T-CELLS IN INDUCTION AND SUPPRESSION OF EXPERIMENTAL AUTOIMMUNE UVEITIS

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Purpose: Experimental autoimmune uveoretinitis (EAU) is a T cell mediated inflammatory autoimmune disease. The role of gamma-delta T cells in EAU is still unknown. We investigated the effect of gamma-delta T cells on the induction and suppression of EAU by oral tolerance in the Lewis rat.
Methods: Neonatal rats were injected with the gamma-delta TCR-specific monoclonal antibody V65 until week 7, when the rats were immunized with retinal autoantigen S-Ag emulsified in CFA. Disease was assessed clinically as well as by histology. For adoptive transfer of suppression, gamma-delta T cells from orally tolerized rats were transfered to naive recipients prior to disease induction.
Results: Perinatal treatment lead to depletion of gamma-delta T cells in peripheral blood, spleen and lymphnodes. We found that the depletion of the gamma-delta TCR$^+$ subpopulation had no influence on the induction of EAU by immunization with S-Ag derived peptide PDSAg.
However, the adoptive transfer of gamma-delta T cells from spleens of rats orally tolerized with S-Ag resulted in reduced incidence and severity of disease, as compared to cells from rats fed with unrelated control peptide.
Conclusions: Gamma-delta T cells are obviously not necessary for the induction of autoimmune uveitis, but rather seem to be important for the suppression of autoimmune responses.

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ANTIGEN-SPECIFIC T CELLS ARE FOUND IN RETINA OF PASSIVELY INDUCED EAU: FLOW CYTOMETRIC ANALYSIS OF PUTATIVE APC WITHIN THE RETINA.

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Purpose: To determine whether retinal antigen-specific CD4$^+$ T cells can be isolated from the retina in a passivily-induced EAU model. We also attempted to isolate and classify by flow cytometry, the cell surface phenotype of microglia in the normal rat retina with a view to identifying putative antigen presenting cells.
Methods: Tracking retinal-antigen CD4$^+$ T cells was performed by flow cytometry in a passivley-induced EAU model in congenic PVG rats with different allotypes for CD45 antigen (CD45^a and CD45^b). Normal rat retinal microglia were isolated and classified using a modification of an isolation technique employing graduated Percoll density gradient cell suspension and flow cytometric phenotypic phenotype criteria used for CNS microglia.
Results: Retinal antigen-specific CD4$^+$CD45^{b+} T cell lines induce EAU in PVG^a rats and these cells can be isloated early in the disease. Retinal microglia can be defined on the basis of CD45^{low}CD11b/c$^+$CD4^{low} cell surface expression. However, constitutive MHC class II expression (present on APC) was confined to a minor population of cells (not microglia) of CD45$^{low/high}$ED2$^+$ phenotype.
Conclusions: Antigen-specific CD4$^+$ T cells can be isolated from the retina early in the disease. The retina also contains a minor population of constitutively expressing MHC class II$^+$ cells which we propose are the counterpart of perivascular macrophages found in the CNS which present antigen to extravasating T cells. The role of parenchymal microglia as APC remains undefined.

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THE ROLE OF THE RETINAL VASCULAR ENDOTHELIUM IN CELLULAR TRAFFICKING IN EAU IN VIVO

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Purpose To define the relative importance of activated leucocytes and retinal endothelial cells in vivo in determining the passage of cells across the blood-retinal barrier (BRB) in EAU.
Methods Spleen cells separated from Lewis rats immunized with 50 μg bovine retinal s-antigen in complete Freund's adjuvant 14 days earlier were stained with the carbocyanine dye - DiI. Stained cells (1x10^7) were injected intravenously into either diseased or normal Lewis rats. After 30 minutes retinal flat mounts were prepared from the recipients and examined by confocal scanning laser microscopy to determine the presence and location of stained cells in relation to retinal vessels.
Results Spleen cells derived from either diseased or normal animals passed freely into the retinal tissues of diseased recipients; by contrast, these cells did not appear in retinas from normal recipients or from immunized animals until there were clinical signs of EAU.
Conclusions The findings implicate the retinal vascular endothelial cell in controlling the passage of leucocytes across the BRB in vivo during EAU and challenge the view that non-specific activation of leucocytes is all that is required in this event.